Facultative and non-facultative sex ratio adjustments in a dimorphic bird species

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Abstract

If parental allocation to each offspring sex has the same cost/benefit ratio, Fisher's hypothesis predicts a sex ratio biased towards the cheaper sex. However, in dimorphic birds there is little evidence for this, especially at hatching. We investigated the pre-fledgling (*i*) sex ratio, (*ii*) body condition and (*iii*) sex-differential mortality in a population of the glossy ibis (*Plegadis falcinellus*), in southern Spain between 2001 and 2011. We defined two age groups for the period between hatching and fledging. We also compared pre-fledgling with the autumn sex ratio. Metabolic rates were estimated by the Doubly Labeled Water (DLW) technique to establish that sons (the bigger sex) were 18% more energy demanding than daughters, and to compute the predicted Fisher's sex ratio (0.465). As population size increased between years, body condition decreased in both sexes, and mortality increased more for daughters than sons prior to fledging. At the same time, the proportion of males among chicks close to fledging increased (average sex ratio: 0.606) while the proportion close to hatching decreased (average sex ratio: 0.434, in line with Fisher's prediction). Furthermore, the proportions of males at fledging and the following autumn were negatively correlated across years. We suggest that, as population density increased and conditions worsened the larger sex had relatively higher survival. These differences in survival produce a shift from a facultative female-biased sex ratio at hatching into a non-facultative male-biased sex ratio of fledglings. Additionally, the excess of males at fledging was counterbalanced by sex-related dispersal during the autumn. Overall, glossy ibis sex ratio is a product of a combination of facultative and non-facultative adjustments triggered by environmental conditions, driven by rapid population growth, and mediated by highly interrelated life-history traits such as body condition, mortality, and dispersal.

Key-words: sex allocation, energy expenditure, Doñana, equal allocation hypothesis, population dynamics, molecular sexing

Introduction

Differential sex allocation occurs when different amounts of resources are devoted to sons and daughters, which can bias the sex ratio, an important life-history trait, at the individual and/or population level. Even though clear-cut boundaries are not well defined (West 2009), sex ratio is usually studied at three different stages: primary (at fertilization), secondary (at birth) and tertiary (at sexual maturity). However, differential mortality or dispersal of sons and daughters can also cause shifts in sex ratio in between these stages.

Since Darwin (Darwin 1871) addressed this topic, several evolutionary theories and empirical studies have been proposed (reviews in Hardy et al. 2002; West 2009). Fisher's landmark equal allocation theory (Fisher 1930) aimed to explain the common occurrence of balanced sex ratios in nature by postulating that, if fitness cost/benefit ratios are not sex dependent, parents should invest the same in daughters and sons. Thus, provided both daughters and sons have the same cost for parents, frequency-dependent selection would offset every deviation from parity and lead to a balanced, evolutionary stable sex ratio. On the other hand, when the sexes require different amounts of resources, selection would skew the sex ratio towards the cheaper sex, if identical parental allocation for each sex was maintained.

Other theories have been developed by considering situations where there are different costs and benefits of male and female offspring, when sex allocation should be adjusted to maximize fitness. There are two main theoretical frameworks: *i*) one postulating that parents should adjust offspring sex ratio depending on predictable sibling cooperation or competition (Local Mate Competition; Hamilton 1967, Local Resource Competition; Clark 1978, Taylor & Bulmer 1980, or Local Resource

Enhancement; Emlen, Emlen & Levin 1986, Lessells & Avery 1987), and *ii*) one proposing that adaptive sex ratio adjustments depend on the specific conditions of parents (Trivers and Willard 1973). In birds, although a number of alternative explanations coexist (reviewed in West 2009), linear (Wiebe and Bortolotti 1992, Appleby et al. 1997, Arroyo 2002) or non-linear (Millon and Bretagnolle 2005) relationships between environmental covariates and sex ratio have often been interpreted as supporting the Trivers & Willard hypothesis.

Given the central role offspring costs play in sex allocation theories, dimorphism has often been considered a proxy for different rearing costs (Trivers and Hare 1976, Clutton-Brock et al. 1985, Magrath et al. 2007), and sexually size dimorphic species have been used as models for testing sex allocation theories (e.g. Stamps 1990; Anderson et al. 1993; Torres & Drummond 1999). Nevertheless, the relationship between sexual size dimorphism and differential costs has rarely been demonstrated (but see Magrath et al. 2007). In species with parental care, sex allocation refers to the resources (time and energy) parents devote to their offspring (Komdeur 2012). As well as dimorphism, differential mortality during the parental care period can alter the costs of each offspring sex, provoking facultative modification in primary sex ratio but also facultative and non-facultative sex ratio variation at the time of fledging (Leigh 1970, West 2009). For instance, if offspring of the larger sex suffer higher mortality then the difference in raising costs between the larger and smaller sex would be reduced (because of shorter parental care time, Komdeur 2012). This might explain why, in dimorphic birds, there is more support for Fisher's prediction at fledging than at hatching (Pen et al. 2000, Benito and González-Solis 2007). Also, if differential mortality is determined by environmental factors and not by facultative manipulation, the sex ratio at fledging can be biased in the opposing manner to that

93 predicted by theory (Dhondt and Hochachka 2001). In such a scenario, sex-related
94 dispersal could provide a facultative mechanism to offset a maladaptive sex ratio.

95 The body condition of chicks can be related to food availability and/or quality,
96 competition for resources, and parasite load. While a direct relationship with these
97 factors can be difficult to be ascertain, these processes can be mediated by population
98 density (reviewed in Brown & Brown 1996). Two opposing hypotheses have been
99 postulated to explain how population density may affect access to resources
100 (reviewed in Brown & Brown 2001): *i*) the enhanced foraging efficiency hypothesis,
101 which predicts that at high density the access to resources is facilitated by some
102 behavioral change (e.g. Ward & Zahavi 1973; Allainé 1990), and *ii*) the increased
103 competition hypothesis which posits that, as population size increases, foraging
104 efficiency declines (e.g. Tella et al. 2001; Dann & Norman 2006). Body condition of
105 chicks can also depend on increasing parasite load (e.g. Merino & Potti 1995) which
106 can increase along with colony size (Brown and Brown 1986, Rózsa et al. 1996).
107 According to the 'Silver Spoon' hypothesis (Grafen 1988), conditions at an early
108 life-stage affect the individual fitness, as confirmed by many empirical studies (e.g.
109 Van de Pol et al. 2006; Stamps 2006). Thus, following the above rationale, the body
110 condition of chicks might change the sex-related costs and benefits (Kruuk et al.
111 1999, Wilkin and Sheldon 2009), and therefore cause adaptive sex ratio adjustments.

112 The present study was centered on sex ratio variation and its underlying causal
113 mechanisms in the Glossy Ibis (*Plegadis falcinellus*), a dimorphic species nesting in
114 Doñana (SW Spain) (Santoro et al. 2010) where it has undergone an explosive
115 population expansion from 7 pairs in 1996 to more than 7,000 pairs by 2011. Only
116 population-level data on sex-ratios were available because the chicks, cared for by

117 both parents, leave the nest and form creches when they are two weeks old (Cramp
118 and Simmons 1977) and collection of brood-level data at an earlier stage was not
119 feasible because of potential disturbance to the breeding colony (Götmark 1992).

120 We aimed to investigate: (*i*) if male chicks had greater energetic requirements than
121 females, as expected from their larger size at fledging, (*ii*) if pre-fledgling sex ratio
122 departed from that expected from simple chromosomal assortment (1:1) or from
123 Fisher's equal allocation theory (according to the estimated energetic requirement of
124 each sex), (*iii*) if pre-fledgling sex ratio, sex-specific body condition or sex-
125 differential mortality depended on colony size or other proxies for the availability of
126 resources, and (*iv*) if the pre-fledgling and autumn sex ratios were inversely related
127 as expected if dispersal counterbalances the excess of one sex amongst offspring.
128 Since time elapsed from hatching can affect the proportion of males by differential
129 mortality, we tested the sex ratio hypotheses by considering pre-fledglings either as a
130 whole or divided into two subgroups according to their age.

Methods

Study Area, Field Data

The "Lucio de la FAO" ("FAO" hereafter) hosts the main Glossy Ibis colony at Doñana, a protected area in SW Spain (Santoro et al. 2010, Ramo et al. 2013). Here, seven pairs settled in 1996 and since then this species has regularly nested in the area, except for three years (1999, 2005 and 2012) when drought events prevented them from breeding owing to an absence of foraging habitat. At the FAO colony, the number of breeding pairs has increased dramatically (more than 2,000 pairs in 2011) and since 2004, additional breeding sites were occupied by this species in Doñana, hosting about 4,000 pairs in 2011 (Ramo et al. 2013).

Chicks were molecularly sexed (Griffiths *et al.* 1998) from samples obtained during ringing operations at the main colony (Santoro *et al.* 2010). Samples were obtained by either 1) pulling of one outer rectrix or 2) taking a sample of blood from the medial metatarsal vein.

Statistical analyses

If sex-differential mortality occurred during the parental care period, then we would expect to find a different sex ratio at hatching and at fledging, and therefore observe differences in sex-ratio between younger and older pre-fledglings. For this reason, we defined two subgroups according to age. As a proxy for an individual's age regardless of sex, we used the wing length, a non dimorphic measure in this species (Figuerola et al. 2006). Wing length has been shown for other bird species to be relatively unaffected by nutritional status and a good proxy for age of chicks (Gaston 1985, Benowitz-fredericks and Kitaysky 2005). We considered the younger group to

be those whose wing length was equal to or shorter than the overall median (sexes combined). Then, ecological hypotheses on sex ratio variation were tested using pre-fledgling sex ratio for the two age groups separately as well as on the whole data set.

All statistical analyses in this study were implemented in R version 3.0.2 (R Development Core Team 2013). The sample size and the number of degrees of freedoms for each hypothesis tested differed according to the data available for the specific set of variables.

DNA-sampling procedure: effect on sex ratio and body condition

Whereas pulling a feather is a straightforward task and requires a short handling time, bleeding a chick is a more time demanding (Magrath *et al.* 2007) and specialized task providing a larger blood sample suitable for other studies. Thus, a systematic tendency to avoid bleeding smaller or less healthy-looking individuals may exist. To check if this was a source of bias for our study, we compared the sex ratio and body condition observed in chicks that were bled against those from which a feather was pulled. We assumed the latter to be completely random with respect to sex and body condition, because all chicks encountered outside nests were ringed, and feathers were taken from as many of these as time allowed.

As a body condition index, we used the Scaled Mass Index (SMI) which has several advantages over alternative methods (Peig and Green 2009, 2010), and which performs well in birds (Bókony et al. 2012, Guillemain et al. 2013). Given that Glossy Ibis chicks are sexually dimorphic (Figuerola *et al.* 2006), the SMI was calculated separately for each sex using measures of body mass and tarsus length (details in Appendix A1).

First, to test the hypothesis that sampling by bleeding biased the sex ratio estimates, we considered a subset of our data drawn from colony visits ("field-sessions" hereafter) in which both sampling procedures were used ($n_{feather}$= 1080, $n_{bleeding}$= 597, $n_{sessions}$= 24, n_{years}= 7). We ran a binomial GLMM (*glmer* function from package *lme4*; Bates et al. 2012) considering the sex ratio (number of males out of total number of sampled chicks) as response variable (*cbind* function; Wilson & Hardy 2002), the year as a random factor and the sampling procedure as an explanatory variable. We also aimed to rule out the possibility that the effect of sampling procedure on sex ratio was due to a procedure-related propensity to select individuals of different ages, for which different sex ratios might exist. Thus, we ran a binomial GLMM considering the sex of an individual sex as response variable, field-sessions nested within year as random effects and the sampling procedure and wing length (a proxy for age) as predictors together with their interaction. We used a subset of data from years for which both sampling procedures had been used, and wing length was measured ($n_{feather}$= 637, $n_{bleeding}$= 459, $n_{sessions}$= 8, n_{years}= 5).

Secondly, to test the effect of sampling procedure on the body condition index we performed a gaussian LMM (*lme* function from package *nlme* ; Pinheiro et al. 2013) on a subset of data for which both bleeding and feather samples were available in each field-session ($n_{feather:females}$= 321, $n_{feather:males}$= 316, $n_{bleeding:females}$= 174, $n_{bleeding:males}$= 285, $n_{sessions}$= 8, n_{years}= 5). We considered the average body condition of each sex for each field-session and sampling procedure as the response variable, year as a random factor, and sex, sampling procedure and their interaction as predictors. The LMM was weighted for the number of replicates from which average values of body condition were calculated for each field-session.

Energetic requirements of male and female chicks

Daily Energy Expenditure (DEE) was estimated using the DLW technique (Butler *et al.* 2004). We fenced a small section of the colony (50 x 50 meters) using a plastic wire mesh (1.5 m high) from May 20th to May 25th 2009. Forty-six chicks (20 females and 26 males) were injected intraperitoneally with 0.5 ml of ^{2}H- and ^{18}O-enriched water (Speakman 1997). All the dose syringes were weighed to four decimal places prior and post administration using an electronic precision balance. After being injected, chicks were retained in shaded and ventilated cardboard boxes during an hour, to allow isotopes to reach equilibrium (Król and Speakman 1999), then a first blood sample was taken and the chicks were released inside the fenced area. A second blood sample was taken after 48/72 hours (fieldwork sessions on 20th, 22nd and 25th May) to estimate the isotope elimination rate and minimize the influence of circadian deviation in DEE (Speakman and Racey 1988). Taking samples over multiple days minimizes the substantial day to day variance in DEE (Speakman et al. 1994, Berteaux et al. 1996). Four individuals were blood-sampled without prior injection to estimate background isotope enrichments of ^{2}H and ^{18}O (Speakman & Racey 1987: method D). Blood samples were immediately heat sealed into 2 x 100µL glass capillaries. Capillaries that contained the blood samples were then vacuum distilled (Nagy 1983), and water from the resulting distillate was used to produce CO_2 and H_2 (methods in Speakman et al. 1990 for CO_2 and Speakman & Król 2005 for H_2). The isotope ratios ^{18}O: ^{16}O and ^{2}H: ^{1}H were analyzed using gas source isotope ratio mass spectrometry (Optima, Micromass IRMS and Isochrom µG, Manchester, UK). Samples were run alongside three lab standards for each isotope (calibrated to International standards) to correct delta values to ppm. Isotope enrichments were converted to values of daily energy expenditure using a single pool

model as recommended for this size of animal by Speakman (1993). There are several alternative approaches for the treatment of evaporative water loss in the calculation (Visser and Schekkerman 1999). We chose the assumption of a fixed evaporation of 25% of the water flux (equation 7.17: Speakman 1997), which has been established to minimize error in a range of conditions (Visser and Schekkerman 1999, Van Trigt et al. 2002).

No knowledge about potential differences in fledging times between sexes exists in this species, and samples were collected from chicks of different ages. For these reasons, we assumed differences between sexes in estimated DEE reflected differences in total expenditure, and considered them as proxies of investment in sons and daughters (Magrath *et al.* 2007).

We tested for sexual differences in DEE using linear regression models with type III sum of squares (function *lm* from package *car*). We used DEE as response variable and sex and wing, or sex and body mass, as predictors. Unlike wing length, body mass is highly dimorphic among Glossy Ibis chicks (Figuerola et al. 2006).

Pre-Fledgling sex ratio: departure from parity and Fisher's hypothesis

According to Fisher's argument, natural selection favors equal allocation in both sexes, hence, it is expected that:

$$nF \times cF = nM \times cM \quad (eq.\ 1)$$

Where cF and cM represent the average costs of raising one female and one male respectively, and nF and nM the number of males and females (adapted from

Charnov 1982). By simple algebra, it can be shown that predicted sex ratio according to *eq. 1* is:

$$\frac{nM}{nM+nF} = \frac{c\bar{F}}{c\bar{F}+c\bar{M}} \quad (eq.\ 2)$$

Thus, in the case where the average costs of rearing a female and a male are identical ($c\bar{F} = c\bar{M}$), the expected sex ratio equals 0.5 and Fisher's argument cannot be distinguished from simple meiotic sex determination (Griffiths et al. 1998, Harvey et al. 2006). On the contrary, if $c\bar{F} \neq c\bar{M}$, then the predicted sex ratio is not balanced and its value can be estimated by *eq. 2*.

We used the average DEE value of females and males as proxies for the average costs of raising daughters and sons respectively, to calculate the predicted sex ratio according to Fisher's equal allocation theory (eq. 2). Then, we computed yearly sex ratio values as the proportion of male fledglings within the total sample of sexed chicks.

We tested if the sex ratios observed in different years deviated from those predicted according to the Fisher' and Random Assortment hypotheses by using a one-sample t-test on the yearly differences between observed and predicted sex ratios.

Environmental covariates and relationship with pre-fledgling sex ratio, body condition and sex-differential mortality

We examined the correlations between breeding population size or the surface area of flooded marshes on the one hand, and the pre-fledgling sex ratio, body condition of each sex, and sex-differential mortality on the other. Marsh area is a measure of the amount of feeding habitat available (see Santoro et al. 2010 for details), and is

dependent on winter rainfall which itself has a positive effect on population growth (Ramo et al. 2013). A preliminary analysis showed that, whereas the yearly average wing length of male chicks increased linearly from 2002 to 2011 ($t = 4.2$, d.f. = 5, $r = 0.88$, $p < 0.05$), that of females did not ($t = 0.98$, d.f. = 5, $r = 0.40$, $p = 0.38$). Thus, assuming that there was no bias from the research staff towards collecting data from older individuals as time progressed, we used the yearly average difference between wing lengths of male and of female chicks as a proxy of sex-differential mortality ("sex-biased mortality" hereafter). Additionally, in cases where we found a significant correlation between body condition and other variables, we tested for a difference between sexes by comparing the two slope coefficients with the Z test (Cohen *et al.* 2003). We did the same to compare slopes between age groups when there was a significant correlation between sex ratio and colony size.

Pre- and post-fledgling sex ratios

We tested the correlation between pre- and post-fledgling sex ratios. Since we aimed to test if the sex ratio close to the fledging time was inversely related to the autumn sex ratio, we tested this relationship on all the pre-fledglings (regardless of age) and those closer to fledging. As estimates of post-fledgling sex ratio, we used estimates from capture-recapture analyses performed in Santoro, Green & Figuerola (2013) on individuals first resighted between October and December of each year.

Results

Effect of DNA sampling procedure on sex ratio and body condition

The probability that an individual was a male was higher among those whose DNA was obtained by bleeding than those which were feather-sampled (slope ± S.E. = 0.5±0.1, z = 4.78, $P < 0.001$). After controlling for the age effect proxied by wing length, the sampling procedure still had an effect on sex of individuals sampled (*wing*, slope ± S.E. = 0.40±0.065, z = 6.23, $P < 0.001$; *sampling procedure*, slope ± S.E. = 0.34±0.12, z = 2.91, $P < 0.01$; *sampling procedure:wing*, slope ± S.E. = 0.19±0.13, z = 1.44, $P = 0.15$). However, we did not find any effect of sampling procedure on body condition estimates after controlling for the effect of sex (*sampling procedure*, slope ± S.E. = 0.02±0.03, t = 0.64, $P = 0.56$; *sex*, slope ± S.E. = -0.06±0.03, t = -1.94, $P < 0.05$; *sampling procedure:sex*, slope ± S.E. = -0.003±0.05, t = -0.07, $P = 0.94$). To avoid any bias due to sampling procedure, further analyses on sex ratio and on sex-differential mortality were performed using only individuals sexed from feather samples.

Daily Energy Expenditure of female and male chicks

Estimated DEE was higher in males than in females (mean ± S.D.: 510.36±15.2 kJ day^{-1} vs. 442.96±13.3 kj day^{-1}) after controlling for wing size (*sex*, estimate ± S.E. = 67.40±20.52, $F_{1,43} = 10.78$, $P < 0.01$; *wing*, slope ± S.E. = 1.39±0.64, $F_{1,43} = 4.7$, $P < 0.05$; Fig. 1). However, such differences disappeared when controlling for body mass (*sex*, slope ± S.E. = 21.64±20.95, $F_{1,43} = 1.07$, $P = 0.31$; *body mass*, slope ±

S.E. = 0.74±0.16, $F_{1,43}$ = 21.8, P < 0.001), which was the best predictor of DEE regardless of individual sex.

Observed sex ratio against predictions from Fisher's and Random Assortment

According to *eqn 2* and average DEE values for female and male chicks, the expected sex ratio according to Fisher's equal allocation theory is 0.465, i.e. skewed towards females. However, over the study period the overall pre-fledging sex ratio was on average male-biased (0.523). This observed sex ratio was significantly higher than that predicted by Fisher (t = 4.42, df = 8, P = 0.002), but not from that predicted by random assortment (t = 1.72, df = 8, P = 0.124). However, for the younger chicks, sex ratio was on average female-biased (0.434), and significantly lower than the balanced sex ratio (t = -3.04, df = 6, P = 0.023), but not different from that predicted by Fisher's hypothesis (t = -1.44, df = 6, P = 0.201). On the contrary, for the older chicks the observed sex ratio was on average male-biased (0.606) and deviated significantly from both a balanced (t = 3.72, df = 6, P = 0.010) and Fisher's sex ratio (t = 4.95, df = 6, P = 0.003).

Relationship between environmental covariates and pre-fledgling sex ratio, body condition and sex-differential mortality

The size of the breeding population of glossy ibis between years at the FAO colony was positively correlated with the overall proportion of males (t = 3.47, df = 7, r = 0.80, P < 0.05) and with sex-biased mortality (t = 3.63, df = 5, r = 0.85, P < 0.05) among pre-fledglings. However, by considering the age groups, we found that with increasing colony size the proportion of males among younger chicks decreased (t = -2.55, df = 5, r = -0.75, P = 0.05), while the opposite occurred among older chicks (t

= 1.98, df = 5, r = 0.66, P = 0.10) (Fig. 2). This difference in sex ratio vs. colony size slopes between age groups was highly significant (z = 3.22, P = 0.001).

In contrast, the size of the breeding population was negatively correlated with body condition (*females*: t = -2.96, df = 8, r = -0.72, $P < 0.05$; *males*: t = -4.11, df = 8, r = -0.82, $P < 0.01$), with no difference in this effect between sexes (z = 1.31, P = 0.19) (Fig. 3).

The surface area of flooded marshes was not related to *overall sex ratio* (t = 1.21, df = 7, r = 0.42, P = 0.26), *sex ratio of younger pre-fledglings* (t = 0.14, df = 5, r = 0.06, P = 0.89), *sex ratio of older pre-fledglings* (t = 1.98, df = 5, r = 0.25, P = 0.58), *sex-biased mortality* (t = 1.22, df = 5, r = 0.48, P = 0.27), *female body condition* (t = -1.88, df = 8, r = -0.55, P = 0.10) or *male body condition* (t = -1.42, df = 8, r = -0.45, P = 0.19).

Relationship between pre- and post-fledgling sex ratio

We found that an increasing proportion of males among pre-fledglings was associated with a decreasing proportion of males among post-fledglings about four months later (t = -2.39, df = 7, r = -0.67, $P < 0.05$). Although the sample size available was reduced by limiting the test to older pre-fledglings, the effect size of this relationship increased (t = -3.15, df = 5, r = -0.82, $P < 0.05$) (Fig. 4).

Discussion

By considering younger and older pre-fledglings, we found sex ratio patterns that would have gone undetected had we not accounted for age differences. In particular: *i*) younger pre-fledglings showed a significant female-biased sex ratio, which agreed

with the Fisher's prediction and was enhanced by colony size increase, whereas *ii*) older pre-fledglings showed a significant male-biased sex ratio and the effect of colony size was the opposite to that recorded for younger birds. Overall, our findings suggest that poor conditions triggered non-facultative sex-differential mortality, leading to maladaptive fledgling sex ratios which in turn were balanced by sex-related post-fledgling dispersal. Our study suggests the co-existence of facultative and non-facultative adjustments triggered by environmental variation, and affecting life-history traits like body condition, differential mortality and sex ratio.

Pre-fledgling sex ratio

If parents manipulate sex ratio during the parental care period, then Fisher's expectations apply at the time of fledging (discussed in Komdeur 2012). This is coherent with two comparative analyses on birds with sexual size dimorphism, which showed that population-level sex ratios tend to be biased towards the smaller sex at fledging (Pen et al. 2000, Benito and González-Solis 2007). However, in our study the sex ratio close to fledgling showed the opposite pattern.

The observed male-bias in sex ratio at fledging might be adaptive if, as the population increased, the cost/benefit ratio of sons over daughters also increased. Thus, parents might skew the sex ratio by changing the sex-related order (Badyaev *et al.* 2002) or by allocating more to sons than to daughters so enhancing higher mortality rates of the latter. However, none of these mechanisms is supported by our findings. On the contrary, we attribute the sex ratio observed close to fledging to non-facultative increased mortality of daughters over sons, determined by environmental variation. There are three lines of evidence that support for this hypothesis. First, it could be argued that the change in wing length difference

between sexes, which we assumed to be a product of sex-differential mortality, may be explained by an active change by parents of the proportion of males amongst first-hatched offspring. However, this alternative explanation is completely inconsistent with the increasing proportion of females close to hatching observed in relation to density, and the simultaneous opposing pattern close to fledging. Second, if differences in mortality were due to parental decisions allocating more to sons than to daughters, we would have expected a sharper decline in body condition as colony size increased in females than in males. However our data show an identical slope for each sex. Third, if facultative manipulation of sex ratio occurs, it should be towards the same sex both at hatching and fledging (see West 2009; Komdeur 2012), and this was not the case in our study.

In a similar result to ours, Great Tit (*Parus major*) showed a bias towards the larger sex at fledging under poor conditions, which was the opposite of that expected under Fisher's hypothesis (Dhondt & Hochachka (2001). The authors argue that, whereas parents might be able to control primary sex ratio, they could not control the fledgling sex ratio. Thus, a parent-offspring conflict may arise under specific conditions, with the larger offspring sex outcompeting the smaller sex, even though this is detrimental for parents' fitness. Furthermore, the average cost of females would be further reduced because of reduced parental care time devoted to this sex (discussed in West 2009), while the average benefit of males might decrease because poor condition may have a more negative impact on the fitness of the larger sex (Millon et al. 2011). If sex-related mortality occurs regardless of parental manipulation, we should then expect an increasing bias towards females at hatching as environmental conditions deteriorate. The same outcome would be expected under Fisherian frequency-dependent selection according to which the rare sex has an

403 intrinsically higher reproductive value (Hardy 2002; West 2009). Our study provides
404 strong support for this prediction.

405 Furthermore, the inverse relationship we found between the fledgling and autumn
406 sex ratios suggests that the excess of males at the end of parental care was
407 counterbalanced by their higher dispersal rate. Our autumn sex ratio estimates
408 originate from a previous capture-recapture analysis (Santoro *et al.* 2013) and refer to
409 the proportion of males among first-resighted individuals, most of which were
410 juveniles. The same pattern would have been observed if members of the majority
411 sex had experienced a higher post-fledgling mortality. However, while it is uneasy to
412 find a sound explanation for the sex- and density-related post-fledgling mortality, it
413 is reasonable to hypothesize that the majority sex dispersed at a higher rate in order
414 to limit intra-sexual competition for mates. This hypothesis is also consistent with the
415 higher recruitment rate of first-year individuals found in another study on this
416 population (Santoro 2014). It is known that the body condition of juveniles is related
417 to their dispersal propensity (e.g. Cristol, Baker & Carbone 1999; Barbraud, Johnson
418 & Bertault 2003). Thus, a possible explanation for our result is that when body
419 condition of both sexes was high (at low population density), females had a higher
420 propensity to disperse than males, whereas when condition was low, the larger size
421 of males conferred a dispersal advantage over females (see also Cristol, Baker &
422 Carbone 1999).

423 Although we have referred to the effect of breeding population size, this was highly
424 correlated to the elapsed number of years since the colony was first established,
425 which may potentially affect other ecological processes (see e.g. Duckworth &
426 Badyaev 2007) whose effects cannot be separated from those of colony size. Our

interpretations rely on an extensive literature linking density-dependence mechanisms to sex allocation patterns (review in Wilson & Hardy 2002; West 2009).

In this study we suggest that the higher dispersal rate of males was a consequence of non-facultative male-bias in sex ratio at fledging. This is not consistent with the Local Resource Competition hypothesis (Clark 1978, Taylor & Bulmer 1980) according to which the higher dispersal propensity of males would have been a cause of facultative sex ratio biasing. However, it is noteworthy that under both premises the fitness of male offspring and parents would be enhanced as a result of reduced competition among relatives. Furthermore, the suggested relationships between deteriorating environmental conditions and sex-related differential mortality and dispersal pose intriguing questions concerning the role of sex ratio balancing within metapopulation dynamics.

Body condition

Early life body condition is known to affect life-history traits of diverse taxa (Stamps 2006) including waterbirds (e.g. Cézilly et al. 1995; Barbraud et al. 2003). Due to the sexual size dimorphism of this species (Figuerola *et al.* 2006), body condition was estimated separately for each sex. Therefore relative differences in SMI between males and females should not be interpreted as sexual differences in body condition (Fig. 1; see Peig & Green 2009, 2010 for more discussion). The similar decline in body condition over time suggests that poor environmental conditions affected both male and female offspring in a similar manner. Density-dependence effects observed on glossy ibis body condition may have been caused by higher rates of disease or parasite loads (reviewed in Poulin 2011), by increasing sibling competition or by food depletion (Brown and Brown 1996, Tella et al. 2001, Forero et al. 2002, Dann

and Norman 2006). The ultimate cause for the decline in body condition, as well as the mechanism responsible for the greater mortality rate in female chicks, are interesting subjects for future research. However, it would be necessary to focus on the different strategies parents may use when faced with poor conditions and, consequently, study sex ratio at the brood-level. Although the larger sex has greater nutritional requirements, and consequently tends to suffer higher mortality (Clutton-Brock et al. 1985, Bortolotti 1986, Arroyo 2002), the opposite pattern can be true if the larger sex outcompetes the smaller when resources are limited (see also Oddie 2000), as has been suggested for the closely related white ibis (*Eudocimus albus*) (Adams and Frederick 2009). In addition, as found in an experimental study on kestrels (*Falco tinnunculus*) (Fargallo *et al.* 2002), the smaller sex may have lower cell-mediated immunity and therefore higher disease susceptibility under food restriction.

Energetic requirements of female and male chicks

Male glossy ibis chicks had higher energetic requirements than females. When mass was used as a covariate in the analysis, this sex effect disappeared, showing that the sex difference in size caused the difference in energy demands. This dominance of size effects on energy demands of different sexes is consistent with studies of other dimorphic species (Scantlebury et al. 2006). Since the DLW experiment was performed on individuals of various ages, we consider our assumption that the ratio between DEE of males and females is representative of the energetic demand throughout the parental care period to be realistic.

Sampling procedure, a warning for colonial species

Working with colonial species is particularly challenging because of the potential disturbance the fieldwork can cause (e.g. Perennou et al. 1996). When collecting our data we were initially unaware of the biases involved in bleeding. With hindsight, it is perhaps not surprising that a research team may unwittingly select larger individuals for a more intense handling procedure. A preference for larger individuals is also likely caused by the greater ease of finding the medial metatarsal vein in these individuals. As a result, DNA samples from bleeding provided a distorted sex ratio biased towards males which most likely was caused by a selection towards larger, not necessarily older, individuals. This issue may have been overlooked in previous avian studies in which chicks are sexually dimorphic and form crèches (especially other colonial birds), and caution is required when interpreting any results on sex ratio that come from blood samples in such birds.

Final remarks

Thanks to an intensive monitoring of a newly established breeding colony, we found substantial support for facultative and non-facultative sex ratio adjustment driven by a rapidly changing population size in glossy ibis. In particular, colony size variation triggered a cascade of ecological processes affecting nestling body condition, sex-related mortality and sex ratio. We also found support for autumn natal dispersal as a functional mechanism to regulate the excess of one sex at the end of the parental care period. Since for logistical reasons we could not collect data at the brood-level, we cannot exclude the possibility that other facultative sex ratio adjustments occurred across groups (West 2009). Nonetheless, this study provides a novel example of the way facultative sex ratio adjustments can respond to biological features of the

497 species (e.g. sexual dimorphism), environmental conditions (e.g. population-size) but
498 also to non-facultative sex ratio changes (e.g. sex-differential mortality).

499

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Figures

Figure 1. Metabolic costs of female and male chicks. a) Linear relationship of female (empty circles - dotted line) and male (full circles - dashed line) chick wing length with Daily Energy Expenditure (DEE) as estimated by a Doubly Labelled Water experiment; b) Linear relationship of chick (regardless of sex) body mass and DEE.

Figure 2. Sex ratios of younger and older chicks plotted against breeding population size. Black circles and the dashed regression line refer to the sex ratio of younger chicks. Black squares and the solid regression line refer to older chicks. The dotted line indicates an even sex ratio.

Figure 3. Linear relationships between breeding population size and: a) pre-fledgling sex ratio, b) sex differential mortality and c) body condition. Pre-fledgling sex ratio is estimated as the yearly average sex ratio (proportion of males). Sex differential mortality is proxied by the yearly difference between the average wing length of male and of female chicks. Body condition estimates were calculated as the scaled mass index of mass versus tarsus, separately for females (empty circles) and males (full circles). Regression lines show the relationship between body condition and breeding population size for each sex.

Figure 4. Relationship between pre- and post-fledgling sex ratio (proportion of males). Post-fledgling estimates are from a previous capture-recapture analysis published in Santoro *et al.* (2013) and refer to a few months later (May-July vs. October-December). Both the linear relationship with the sex ratio of pre-fledglings

regardless of age (triangles, solid line), and with the sex ratio of older pre-fledglings (squares, dashed line) are shown.